CHRONIC ETHANOL CONSUMPTION INDUCES HYPOMOTILITY IN THE PORTAL VEIN OF SARDINIAN ALCOHOL-PREFERRING RATS

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Abstract — In order to study the physiopathological effects of chronic ethanol intake on the smooth muscle of the vascular system, we have assessed the length–tension relationship in isolated portal veins of Sardinian alcohol-preferring (sP) rats. Significant differences in motor performance were found between sP naive and sP rats exposed to ethanol consumption (12% w/v) for 48 weeks. Isolated portal veins of sP rats which consumed ethanol chronically showed a marked decrease of spontaneous and KCl-induced contraction waves when compared to sP naive rats. At optimum length (140% L0) for maximal contractile performance, the mean amplitude wave in the portal veins of sP drinker rats was about five times less than in sP naive veins. Furthermore, in the veins of sP drinkers, the active curve showed lower values of tension at each elongation of the vascular segment, the maximum value of active tension (7.32 ± 0.54 mN) represented a reduction in amplitude of about 32% with respect to sP naive veins. These results indicate that long-term ethanol consumption impairs portal vein motility.

INTRODUCTION

Chronic alcohol consumption can induce morphological and functional alterations in different organs and tissues of animals and man (Lieber et al., 1975; Fadda and Rossetti, 1998). Animals cannot drink large amounts of ethanol spontaneously, but can be easily intoxicated by forced ethanol administration. It is possible to study the effects of chronic alcohol consumption, on different organs and tissues, by using rats which have been bred selectively for high ethanol preference. By breeding heavy and low alcohol drinkers from a heterogeneous population of rats, we have obtained two rat lines, Sardinian alcohol-preferring (sP) and Sardinian alcohol non-preferring (sNP) (Fadda et al., 1989). We found, after 10 generations of out-breeding of rats, a highly significant difference in ethanol consumption between the two lines.

A considerable number of studies have been made on these selected lines of sP and sNP rats, in order to investigate physiological and biochemical differences between them (see Gessa et al., 1991 and Colombo et al., 1997 for reviews).

With the aim of studying the physiopathological effects of ethanol on the smooth muscle of the vascular system, we investigated the mechanical properties in terms of length–tension relationships in sP naive and drinking rats. The length–tension relationship is a primary property of skeletal, cardiac, and many smooth muscles. It expresses the relationship between the force developed by the muscle when it is stimulated under isometric conditions and the length of the muscle at the time of stimulation.

In the present investigation we report, in quantitative terms, the active and passive mechanical properties determined in isolated portal veins of sP naive and sP drinkers which consumed ethanol (12% w/v) for 48 weeks, in a free choice with water.

METHODS

Animals

Ten sP male rats were used for this study. Animals from the 38th generation were approximately 3 months old and weighed 200–250 g at the start of the experiment. Rats were maintained at 22°C and 60% relative humidity, on a 12 h light–12 h dark cycle (lights on at 07:00). The animals were divided into two groups: (1) five sP ethanol naive rats (sP-N) which had food and water freely available throughout the entire experimental period (48 weeks); (2)

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five sP drinking rats (sP-D), maintained under the same conditions as the first group, but with free choice between tap water and 12% (w/v) ethanol for the same period of time; the mean amount of alcohol consumed during the 48-week period was 6.94 ± 1.08 g/kg/day.

At the end of the treatment, the weights of rats were 550–585 g with no significant difference between the sP-N and sP-D groups. Using deep ether anaesthesia, the abdomen was opened by a parasagittal incision and, under a stereomicroscope (40 x Wild M 313), the portal vein (7 mm length in situ) was carefully dissected from the surrounding tissue and then cut at the level of the gastroepiploic vein and its bifurcation at the liver hilum. The vein was placed in an organ bath (10 ml) containing Krebs solution with the following mM composition: NaCl (118), KCl (4.70), CaCl₂ (2.25), MgSO₄ (1.64), NaHCO₃ (24.88), glucose (5.55). The Krebs solution was bubbled continuously with O₂ 95%–CO₂ 5% (at pH 7.4) and kept at a constant temperature of 35°C. The preparation was mounted vertically and connected to a Grass FT 03 force-displacement transducer. The transducer was mounted on a moving support, allowing minimal length increments of 5 μm. During an equilibration period of about 1 h, the portal vein was gradually stretched until it reached its in situ length. This length was measured using a stereomicroscope equipped with an ocular micrometer, and was defined as the resting length (L₀). When L₀ had been assessed, the portal vein was allowed to equilibrate for about 30 min, by which time spontaneous contractile activity had developed. Before elongation of the portal vein began, maximal active tension was determined by exposing the preparation to 100 mM KCl at 20-min intervals in order to obtain reproducible and persistent contractions. Contact was allowed for 3 min and was followed by repeated washing. Active KCl-stimulated tension was determined at each increment in length of the portal vein. To obtain length–tension responses, the portal vein was stretched by gradual increments of 700 μm (10% of the in situ length) from 100% to 170% L₀. The vein was allowed to equilibrate for 10 min after each increment in length to allow for stress–relaxation. At each length, the passive force exerted by the vein due to stretching, the active force developed in response to 100 mM KCl and spontaneous phasic contractions were recorded by the force transducer. The transducer output was amplified and displayed continuously on a moving paper trace using a pen recorder.

Analysis of data

Length–tension curve determinations. Passive and active length–tension relations were determined in the five sP-N and five sP-D rats. At each increment in the length of the portal vein, the resting tension was measured from the baseline determined at 100% L₀ in the rest period between spontaneous contractions, whereas the active tension was obtained from the amplitude of 100 mM KCl-stimulated contractions recorded at each increment in length.

Spontaneous contractile activity. At each increment in length of the portal vein segment, spontaneous contractile activity was recorded at a high velocity (50 mm × min⁻¹) of recording paper for at least 10 min. During the 10-min control period, and during 5 min subsequent to the stretching, the amplitude of each spontaneous force wave was measured. The frequency of contractile waves was evaluated by computing the number of waves in a 10-min period and is reported as cycles × min⁻¹.

Data are presented as means ± SEM, and the statistical significance was analysed using Student’s t-test.

RESULTS

Figure 1 shows the effects of increments in length of the portal vein between 100% L₀ and 170% L₀. The original tracings illustrate the spontaneous motor activity superimposed on the resting tension and the maximal active tension induced by 100 mM KCl at 100%, 120%, 150%, and 170% L₀ of elongation; the recordings were taken from two preparations of portal vein isolated from sP-N and sP-D rats.

Spontaneous motor activity characteristics

In sP-N control animals, at the initial length (100% L₀), the spontaneous activity of the longitudinal smooth muscle of the portal vein in standard Krebs solution was characterized by a pattern of uniform phasic contractions, which occurred with regular amplitude and frequency. Recordings of this activity during the control period (100% L₀) are illustrated in Fig. 1. The stretch enhanced both the amplitude and frequency of the
Fig. 1. Selected experimental tracings. Tracings show the effect of increasing length (arrows) on resting tension, spontaneous phasic activity and maximal active tension induced by the addition of 100 mM KCl (+) to the organ bath on two isolated portal veins from Sardinian alcohol-preferring non-drinking (sP-N) and Sardinian alcohol-preferring drinking (sP-D) rats. The broken line represents the resting tension recorded at 100% L₀.

contraction waves at each degree of elongation. The shape of the waves was regular during the entire length–tension determination, indicating good synchrony of the contracting waves. Marked steady changes in the spontaneous mechanical activity of smooth muscle were recorded in sP-D rats at resting tension (100% L₀). In these animals, the amplitude of the phasic contractions decreased considerably, whereas the frequency of contraction increased. As shown in Fig. 1, this disordered motor pattern became more evident during the elongation of the portal vein, indicating a certain degree of desynchronization of activity, more evident during the length–tension responses. The quantitative relations between each degree of stretch and the mechanical parameters which characterize the spontaneous motor activity in sP-N and sP-D portal veins are shown in Fig. 2. In the sP-N portal vein, the phasic activity increased together with the extension of the length of preparation up to 140% L₀. Beyond this length, the force declined. Significant (P < 0.05) differences were found between sP-N and sP-D rats. SP-D portal veins showed a marked decrease of amplitude waves. At optimum length (140% L₀) for maximal contractile performance, the mean amplitude wave was about five times less than in sP-N veins, whereas, as far as the frequency was concerned, the sP-D portal vein showed high contraction frequency, with values three to five times higher than the sP-N portal vein.

Length–tension relationships

The average results of length–tension relationships obtained in all rats are illustrated in Fig. 3. In sP-N rats, the passive tension curves obtained in portal veins isolated from their afferent and efferent nervous connections are curvilinear, convexing towards the length axis at about 130% L₀. In this tract of the curve, the relation between length and tension was nearly linear. With further increments in length, the slope of the passive tension curve shifted to the left and reached 9.3 ± 1.2 mN at 170% L₀. The active length–tension
Fig. 2. Effects of varying amount of stretching on spontaneous phasic contraction waves in Sardinian alcohol-preferring non-drinking (sP-N) and Sardinian alcohol-preferring drinking (sP-D) rat portal vein. Stretching varied between 100% and 170% L₀. Effects were examined on amplitude (A) and frequency (B). Points are means ± SEM for five experiments.

Fig. 3. Length–tension relationship for Sardinian alcohol-preferring non-drinking (sP-N) and Sardinian alcohol-preferring drinking (sP-D) rat portal vein. Tissue length is expressed as percentage of the in situ length. Active tension was obtained by the addition of 100 mM KCl to the organ bath at each increment in length. Points are means ± SEM for five experiments.

curve, obtained from the mean amplitude of the KCl-induced contractions, increased as the stretch was extended. The approximate optimal length for the maximal active force development was found in these animals at 140% L₀, whereas the amplitude value of active force was 9.88 ± 1.39 mN.

In sP-D rats (Fig. 3), the length–resting tension curve was similar to that obtained in sP-N rats, whereas significant differences (P < 0.05) were found for the active tension values.

In the portal vein of sP-D rats, evident anomalies in length–resting tension relationships were found. In fact, the entire length–active tension curve was depressed. The marked decrease of the curve at all length increments of vascular segments was significant (P < 0.05) when compared to sP-N veins. The active curve showed reduced tension values at each elongation of the vascular segment. Furthermore, the maximal value of active tension (7.32 ± 0.54 mN) was found at higher values of length (170% L₀), and
presented a reduction in amplitude value of about 32% when compared to those obtained in sP-N animals.

**DISCUSSION**

From the results obtained in these experiments, we observed that motility of the portal vein was affected by chronic ingestion of alcohol. Indeed, sP-D rats showed a desynchronized spontaneous phasic activity, with low amplitude and high frequency and a loss of active force, when compared to sP-N rats.

Two types of spontaneous motor pattern have been recorded in isolated portal veins of different animal species. The more usual motor pattern is characterized by coordinated force waves with regular amplitude, duration, and frequency. Excitation is a myogenic event (Johansson and Ljung, 1967), which initiates in a part of the tissue and spreads electrotonically from cell to cell by means of extracellular junctions (‘nexus’). This results in a synchronized activation (Ljung, 1970). Funaki and Bohr (1964) observed a gradual depolarization in single muscle cells in the portal vein of rats, which led to a burst of action potentials. The areas where the muscular cells exhibit this behaviour might serve as pacemakers, provided that their activity is conducted by specialized ‘myo-muscular junctions’ (nexus) to other parts of the muscle (Johansson and Ljung, 1968; Ljung, 1970). Nexus have been found in several different blood vessels (Rhodin, 1967; Somlyo and Somlyo, 1968). In addition, microscopic studies of the portal vein of rats showed nexus between adjacent muscle cell membranes (Funaki and Bohr, 1964; Barr et al., 1968; Dewey and Barr, 1968; Ljung, 1970). Contraction waves similar to those presented by normal Wistar rats have been recorded in sP-N rats, indicating the absence of functional anomalies in our specially bred animals.

The second, and unusual, type of motor activity which has been recorded is characterized by scattered motor patterns with complex force waves, irregular in amplitude, duration, and frequency (Hermesmeyer, 1973; Mancinelli, 1993). In the portal veins of sP-D rats this activity alone was observed. One possible explanation could be that the frequency of contraction is induced by several active pacemaker areas (Hermesmeyer, 1973). The rate of burst formation from each zone and the amplitude and duration of the mechanical response, might be determined by the degree of synchronization of the portal vein smooth muscle activity (Biamino and Kruckenberg, 1969). This synchronization might depend on the integrity of the intercellular junctions. The marked increase in frequency of contraction waves recorded in sP-D rats suggested a breakdown in the conduction of action potentials at the level of myo-muscular junctions, inducing irregular motor patterns due to several active pacemaker areas (Hermesmeyer, 1973). Furthermore, the reduction of spontaneous phasic and KCl-induced contractions should indicate an effect of ethanol on the smooth muscle contractile mechanisms of the portal vein.

The effect observed on smooth muscle is similar to the acute effect of ethanol in the small intestine of dogs (Lu et al., 1997). These researchers reported that alcohol, in vitro, dose-dependently decreased the amplitude of the contraction waves, hyperpolarized the resting membrane potential, and decreased the amplitude of the slow wave of circular smooth muscle of the small intestine. These observations may be due to the acute effect of ethanol on plasma membranes. Indeed, ethanol can affect membrane organization, the function of membrane-bound enzymes, enzymes and proteins involved in signal transduction, ion channels, receptor-coupled ionophores, carrier proteins (for reviews, see Diamond and Gordon, 1997; Lovinger, 1997), and can alter membrane microdomains that determine protein–membrane and protein–ligand interactions. Ethanol acts directly on membrane proteins, producing conformational changes that alter their function; moreover this action is evident at pharmacologically relevant concentrations of ethanol (Franks and Lieb, 1986; Li et al., 1994; Peoples and Weight, 1995; Lovinger, 1997). It is possible that, in sP drinking rats, ethanol in the portal vein reaches concentrations that can impair the plasmatic membranes, and that this disturbance becomes persistent after long-term ethanol consumption.

The exact physiological significance of spontaneous motor activity of the portal vein remains to be determined. It is possible that longitudinal muscular fibres may allow the segment of vein to contract in a coordinated fashion. Spontaneous contractions might then act either as a pump to assist venous return, or as a sustained contraction trapping blood in the visceral region. However, our
data indicate that both functions are compromised in sP-D rats.

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REFERENCES


